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**Discrimination and Distribution of Two Tropical Short-Finned Eels  
(*Anguilla bicolor bicolor* and *Anguilla bicolor pacifica*) in the Indonesian Waters**

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**Abstract** A total of 272 glass eel specimens of the two tropical short-finned eels (*Anguilla bicolor bicolor* and *Anguilla bicolor pacifica*) were collected around Indonesian waters from 2004 to 2007. Field sampling was conducted in 11 estuaries (Krueng Aceh, Air Kertaun, Cibaliung, Citanduy, Pacitan, Palu, Dumoga, Poigar, Bone Bolango, Akelamo, and Pami River) that spread out from western to eastern Indonesian archipelagos. Objectives of the study were to understand subspecies discrimination and distribution as well as population study of the tropical short-finned eel species (*Anguilla bicolor*) in the Indonesian waters based on morphological analyses of body measurements and vertebrae counts. Although morphological key characters of ano-dorsal length as a percentage of total length ( $-2.13 \sim 3.92$ ) and number of ano-dorsal vertebrae ( $-3 \sim 4$ ) overlap between subspecies, specimens were assigned to one of two subspecies based on their reported range of distribution from the western Sumatera Island to the southern Jawa Island (*A. bicolor bicolor*) and from the western Sulawesi Island to the western Papua Island (*A. bicolor pacifica*). Cluster analyses based on Euclidean distance separated the tropical short-finned eel species into two major clades. The first clade consisted of subspecies of *A. bicolor bicolor* from Cibaliung, Air Kertaun, and Pacitan River estuaries. The second clade consisted of subspecies of *A. bicolor pacifica* from Palu, Dumoga, Poigar, Bone Bolango, and Akelamo River estuaries. Interestingly, *A. bicolor bicolor* from Citanduy River estuary (Jawa Island) nested in the second clade. The results suggested that a single tropical short-finned eel species, *A. bicolor*, inhabit in the Indonesian Waters. However the species was ecologically and biologically separated into two clade of subspecies, i.e. a Western Indonesian Clade of *A. bicolor bicolor* derived from Indian Ocean and an Eastern Indonesian Clade of *A. bicolor pacifica* derived from Pacific Ocean.

**Key words:** tropical short-finned eels, *Anguilla bicolor bicolor*, *Anguilla bicolor pacifica*, Western Indonesian Clade, Eastern Indonesian Clade

### Introduction

About 16 species of anguillid eel were reported to inhabit the world, e.g. *Anguilla celebesensis*, *A. marmorata*, *A. borneensis*, *A. interioris*, *A. megastoma*, *A. obscura*, *A. bicolor*, *A. nebulosa*, *A. japonica*, *A. anguilla*, *A. rostrata*, *A. dieffenbachii*, *A. reinhardtii*, *A. australis*, *A. mossambica*, and *A. luzonensis* (Ege, 1939; Jespersen, 1942; Tsukamoto and Aoyama, 1998; Arai *et al.*, 1999a; Aoyama *et al.*, 2001 and 2003; Minegishi *et al.*, 2005; Sugeha *et al.*, 2001a, b and 2008a, b, c; Watanabe *et al.*, 2005a, b and 2009). Three species among them (*A. bicolor*, *A. nebulosa*, *A. australis*) consist of two subspecies, namely *A. b. bicolor* and *A. b. pacifica* (Ege, 1939; Aoyama *et al.*, 2001 and 2003; Watanabe *et al.*, 2005a, b; Minegishi *et al.*, 2001 and 2005; Sugeha *et al.*, 2008a, b, c), *A. n. nebulosa* and *A. n. labiata* (Ege, 1939; Aoyama *et al.*, 2001; Watanabe *et al.*, 2005a, b; Minegishi *et al.*, 2001 and 2005), and *A. a. australis* and *A. a. schmidtii* (Aoyama *et al.*, 2001; Watanabe *et al.*, 2005a, b; Minegishi *et al.*, 2005), respectively. The short-finned eel, *A. australis*, was reported to inhabit in subtropical region of the southern hemisphere especially in the waters around Australian continent and New Zealand (Ege, 1939; Jespersen, 1942; Jellyman, 1977; Todd, 1981; Sloane, 1984; Chisnall and Kalish, 1993; Arai *et al.*, 1999d; Shiao *et al.*, 2002; Aoyama *et al.*, 2001; Watanabe *et al.*, 2005a, b; Minegishi *et al.*, 2005). The long-finned eel, *A. nebulosa*, was reported to inhabit the tropical region

mostly in the islands around the Indian Ocean (Ege, 1939; Jespersen, 1942; Aoyama *et al.*, 2001, 2003, and 2007; Watanabe *et al.*, 2005a, b; Minegishi *et al.*, 2005). The short-finned eel, *A. bicolor*, was reported to inhabit the sub tropical to the tropical region mostly in the waters around Indonesian archipelagos (Ege, 1939; Jespersen, 1942; Tzeng and Tabeta, 1983; Arai *et al.*, 1999a, b, and c; Setiawan *et al.*, 2001; Aoyama *et al.*, 2001, 2003, and 2007; Watanabe *et al.*, 2005a, b; Kuroki *et al.*, 2006 and 2007; Sugeha *et al.*, 2001a, b and 2008a, b, c).

Indonesian waters are known as the center of distribution of the tropical anguillid eels in the world. Some authors reported the occurrence of five to seven species and sub species of anguillid eels in the waters around Indonesian archipelagos, *e. q.* *A. nebulosa nebulosa*, *A. celebesensis*, *A. marmorata*, *A. borneensis*, *A. interioris*, *A. bicolor bicolor*, and *A. bicolor pacifica* (Ege, 1939; Jespersen, 1942, Aoyama *et al.*, 2001, 2003 and 2007; Watanabe *et al.*, 2005a, b; Minegishi *et al.*, 2005, Wouthuyzen *et al.*, 2009). However the most recent study on the species diversity, geographic distribution, and abundance of tropical anguillid eels in the Indonesian waters suggested that nine species and subspecies inhabit the region, *e. q.* *A. celebesensis*, *A. marmorata*, *A. borneensis*, *A. interioris*, *A. obscura*, *A. bicolor bicolor*, *A. bicolor pacifica*, *A. nebulosa nebulosa*, and *A. megastoma* (Sugeha *et al.*, 2008a, b, c). Those species and subspecies were distributed from the west, the central, to the east region of Indonesian waters in different timing and pattern of abundance that regulated by seasonal tropical monsoon (Sugeha *et al.*, 2008a, b, c). In that study, the highest diversity occurred in central Indonesian waters where five species were found including four long-finned eel species (*A. marmorata*, *A. celebesensis*, *A. borneensis*, *A. interioris*) and one short-finned eel species (*A. bicolor pacifica*). The others species were mostly found in the western Indonesian waters (*A. nebulosa nebulosa* and *A. bicolor bicolor*) and in the eastern Indonesian waters (*A. obscura*, and *A. megastoma*) (Sugeha *et al.*, 2008a).

The short-finned eel species, *A. bicolor*, could be separated from long-finned eel species based on a key morphological character of ano-dorsal length as a percentage of total length (ADL/%TL) and number of ano-dorsal vertebrae (ADV) at juvenile stage (Tabeta *et al.*, 1976; Tzeng & Tabeta, 1983; Sugeha *et al.*, 2001a and 2008b, c). However, the two subspecies of *A. bicolor* (*A. bicolor bicolor* and *A. bicolor pacifica*) were present in the Indonesian waters based on their distribution range (Ege, 1939; Jespersen, 1942; Watanabe *et al.*, 2005a,b; Aoyama *et al.*, 2001, 2003 and 2007; Sugeha *et al.*, 2008a, b, c) and genetic study (Minegishi *et al.*, 2001; Watanabe *et al.*, 2005b; Aoyama *et al.*, 2001, 2003 and 2007; Jamandre *et al.*, 2007; Sugeha *et al.*, 2008a, b, c). Based on morphology, genetics, and geographic distribution, Sugeha *et al.* (2008a, b, c) recorded a simultaneous catch of *A. bicolor bicolor* in the western Indonesian waters (from Sumatera to the Jawa Islands) and *A. bicolor pacifica* in the central (Sulawesi Island) and east region of Indonesian waters (from Halmahera and Papua Islands). In that study the authors found that the *A. bicolor bicolor* dominated the catch in the western region while *A. bicolor pacifica* were dominated in the central region and were least dominant in the eastern regions, compared to the other anguillid eel species collected.

The study of Sugeha *et al.* (2008a, b, and c) validated the occurrence of the two subspecies in Indonesian waters and provided means for recognizing and separating the two subspecies of glass eels in the region. Sympatric distribution between and within *Anguilla* species (Aoyama *et al.*, 2003 and 2007; Gagnaire *et al.*, 2007; Sugeha *et al.*, 2008a) and overlapped population structure within species (Minegishi *et al.*, 2008) and or subspecies (Aoyama *et al.*, 2007; Jamandre *et al.*, 2007) are highly possible in the Indonesian waters and surrounding area. This is especially true in the central Indonesian Seas that are known as the most complicated bio-geographic region in the world due to the complex oceanography of Indonesian Through flow (Godfrey, 1996), thermocline (Ilahude and Gordon, 1996), tidal transport and mixing (Hatayama *et al.*, 1996) and the unique bio-ecological conditions of Wallace Line (Barber *et al.*, 2000, Tomascik *et al.*, 1997) and Weber Line (Tomascik *et*

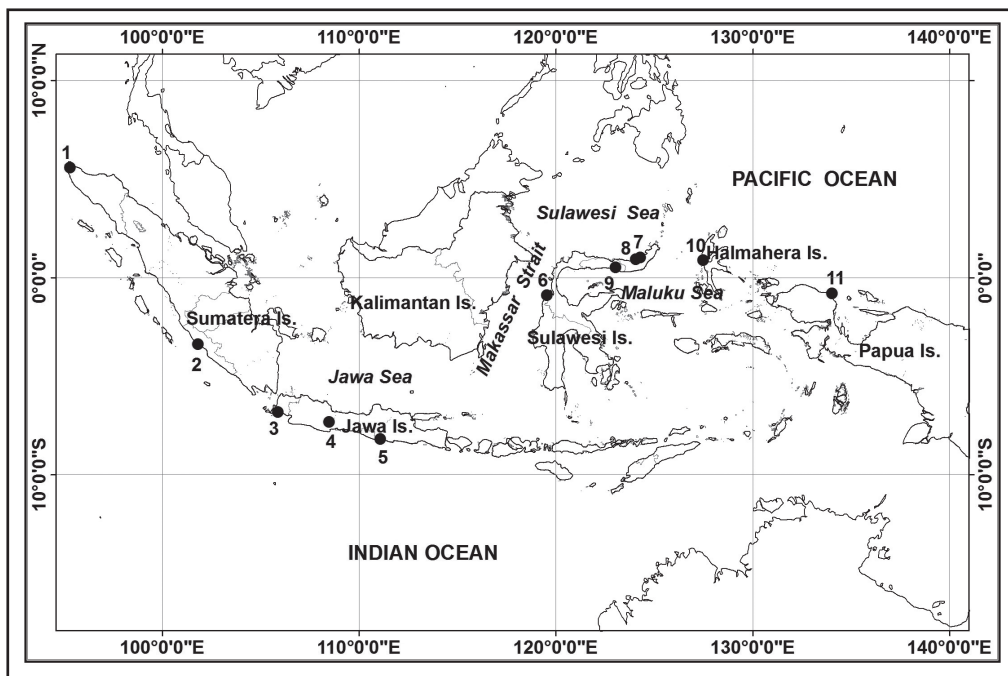
*al.*, 1997). It is important to know whether subspecies of the tropical short-finned eel, *A. bicolor*, in the Indonesian waters consists of single or multiple populations, as was examined recently for *A. bicolor* in Indian Ocean (Watanabe *et al.*, 2005b) and Philippine waters (Jamandre *et al.*, 2007) and for *A. marmorata* in Indonesian waters (Minegishi *et al.*, 2008).

Knowledge on the population structure of the anguillid mainly for temperate eel species, i.e. *A. japonica* (Ishikawa *et al.*, 2004; Tseng *et al.*, 2006; Maes *et al.*, 2006), *A. rostrata* (Maes *et al.*, 2006), and *A. anguilla* (Daemen *et al.*, 2001; Wirth and Bernatchez, 2001; Maes *et al.*, 2006). However, only few studies have been done on the population structure of the tropical eel species, i.e. *A. marmorata* (Robinet *et al.*, 2002; Ishikawa *et al.*, 2004; Maes *et al.*, 2006; Minegishi *et al.*, 2008) and *A. bicolor bicolor* (Watanabe *et al.*, 2005). Therefore, we proposed to study the discrimination and distribution of the short-finned eel, *A. bicolor*, in the Indonesian waters. The objective of this study was to better understand subspecies discrimination and distribution as well as population study of the tropical short-finned eel species (*A. bicolor*) in the Indonesian waters based on morphological analyses of body measurements and vertebrae counts.

## Materials and methods

### Sample collection

Glass eels were collected at the mouth of 11 estuaries (Krueng Aceh, Air Kertaun, Cibaliung, Citanduy, Pacitan, Palu, Dumoga, Poigar, Bone Bolango, Akelamo, and Pami River Estuaries) covering western to eastern Indonesian archipelagos (Figure 1). Glass eel sampling was conducted on the three dates of new moon in 2004 (Citanduy, Pacitan, Dumoga, Poigar, Palu, Bone Bolango Estuaries), six dates of new moon in 2005 (Cibaliung, Palu, Poigar estuaries) and in 2006 (Krueng



**Fig. 1.** Map of the sampling locations of tropical anguillid glass eels, *Anguilla bicolor*, in the Indonesian Waters.

1. Krueng Aceh Estuary; 2. Air Kertaun Estuary; 3. Cibaliung Estuary; 4. Citanduy Estuary; 5. Pacitan Estuary; 6. Palu Estuary; 7. Dumoga Estuary; 8. Poigar Estuary; 9. Bone Bolango Estuary; 10. Akelamo Estuary; 11. Pami Estuary

Aceh, Air Kertaun, Palu, Akelamo, Pami Estuaries) and twelve dates of new moon from May 2006 to April 2007 (Palu Estuary). Method of line transects using triangular scoop nets was done in each location (see Sugeha *et al.*, 2001a and 2008a, b, c). The glass eels were fixed in 10% formalin just after capture and transported to the laboratory for future analyses.

### **Morphological analyses**

A total of 272 glass eel specimen of *A. bicolor* were collected. Total length (TL), *pre-dorsal* length (PDL), and *ano-dorsal* length (ADL) were measured to the nearest 0.1mm (Sugeha *et al.*, 2001a, b and 2008b, c). Pigmentation stage was determined according to Bertin (1956). A part of samples then was stained with alizarin-red solution, and total *vertebrae* (TV), *pre-dorsal vertebrae* (PDV), *pre-anal vertebrae* (PAV), *abdominal vertebrae* (ABV), and *ano-dorsal vertebrae* (ADV) were counted according to Tabeta *et al.* (1976), Tzeng & Tabeta (1983), and Sugeha *et al.* (2001a, b and 2008b, c). Species identification based on the *ano-dorsal* length as a percentage of total length (ADL/%TL) and the number of *ano-dorsal vertebrae* (ADV) as reported by Tabeta *et al.* (1976), Tzeng & Tabeta (1983), and Sugeha *et al.* (2001a, b and 2008b, c).

### **Population study analyses**

Population study of the tropical short-finned eels was analyzed using morphometric data. A dendrogram of separated populations was constructed using Primer 6.0. We carried out cluster analysis based on Euclidean distance with a classification technique using Hierarchical Agglomerative by group average linking of the data of body length measurements.

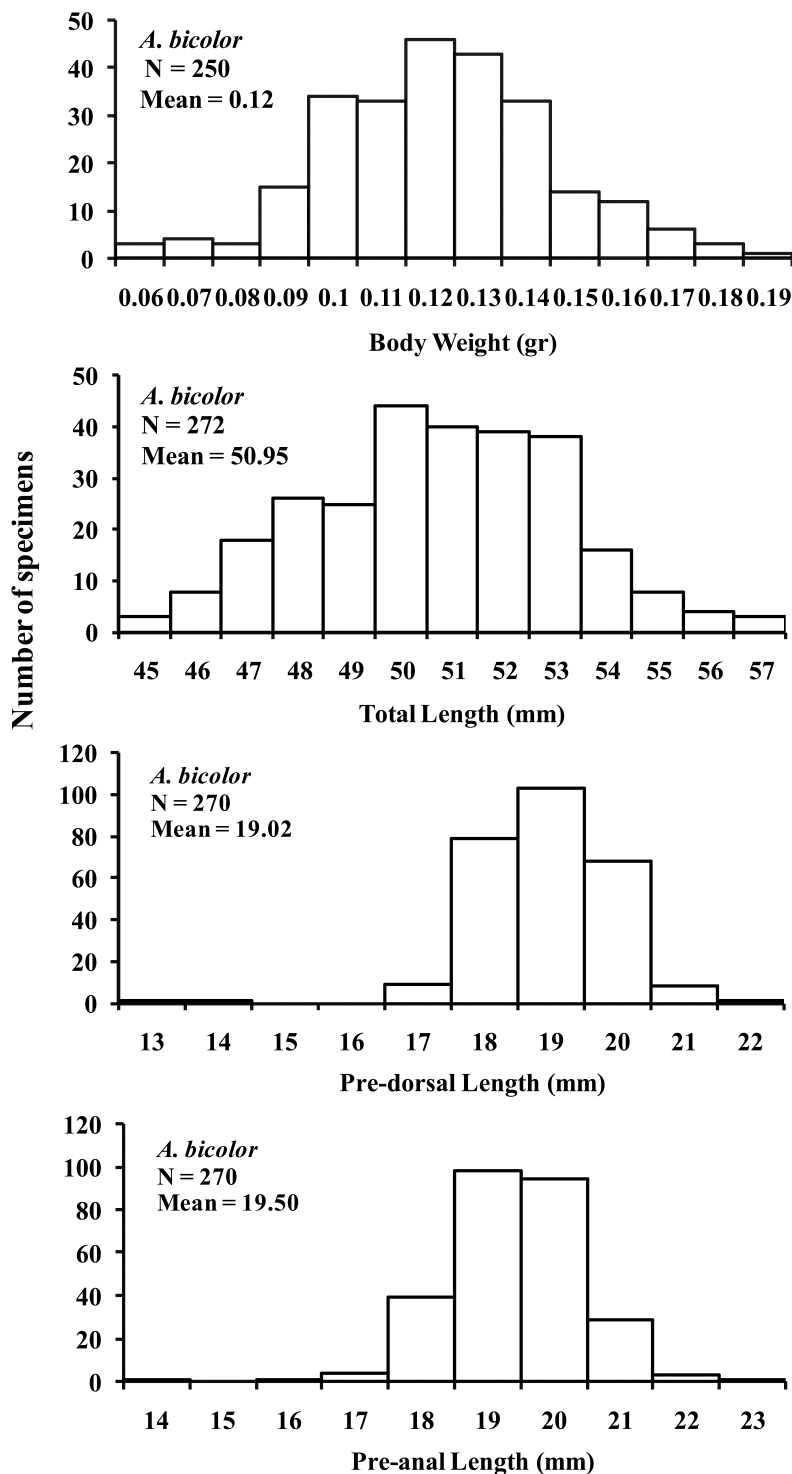
## **Results**

### **Morphological characters of the tropical short-finned eel *Anguilla bicolor***

Glass eel specimens of short-finned eels were relatively smaller than the long-finned glass eels. The short-finned eels could be distinguished from the long finned eels by presence of melanophores that concentrated on the caudal only with none on the body (Tabeta *et al.*, 1976; Tzeng & Tabeta, 1983, Sugeha *et al.*, 2001a, b).

Pigmentation development of the tropical short-finned eels was in the earliest stage compared to the tropical long-finned glass eels. Based on observation of pigmentation development from 264 specimens of the tropical short-finned eels collected, it was found that pigmentation development was dominated by stage VA (93.18%) then stage VB (6.8%). Pigmentation stage VA was characterized by the appearance of pigment spots in the caudal region only while pigmentation stage VB is recognized by the appearance of pigment spots in the caudal and in the skull but none in the body (Bertin, 1956; Sugeha *et al.*, 2001a). The results suggest that glass eel stage captured was just finishing metamorphosis, and just entering the freshwater area.

Total body weight of short-finned eels collected in the present study ranged from 0.06 to 0.19gr ( $0.12 \pm 0.02$ ) (parenthetic values here and elsewhere are mean  $\pm$  one standard deviation), total body length (TL) was 45 to 57mm ( $50.95 \pm 2.48$ ), *pre-dorsal* length (PDL) was 13 to 22mm ( $19.02 \pm 1.03$ ), and *pre-anal* length (PAL) was 14 to 23mm ( $19.50 \pm 1.07$ ) (Figure 2). Total number of *vertebrae* (TV), *pre-dorsal vertebrae* (PDV), *pre-anal vertebrae* (PAV), *ano-dorsal vertebrae* (ADV), and *abdominal vertebrae* (ABV) of the short-finned eels specimens also shown a great range of distribution, *e. q.*, 100 to 113 ( $106.5 \pm 4.82$ ), 30 to 40 ( $35 \pm 3.32$ ), 31 to 39 ( $35 \pm 2.74$ ), and 40 to 45 ( $42.5 \pm 1.87$ ), respectively (Figure 3). Beside the appearance of pigment melanin concentrated in the caudal region and not in the body, based on the characters of ADL/%TL and ADV, it was determined that all 272 specimens of tropical short-finned eels belong to the short-finned eel, *A. bicolor*, with a wide range of ADL/%TL and ADV from -2 to 3 and from -3 to 4, respectively (Sugeha *et al.*, 2001a).



**Fig. 2.** Frequency distribution of body weight, total length, *pre-dorsal* length, and *pre-anal* length of the tropical short-finned eels, *Anguilla bicolor*, collected in the Indonesian Waters.

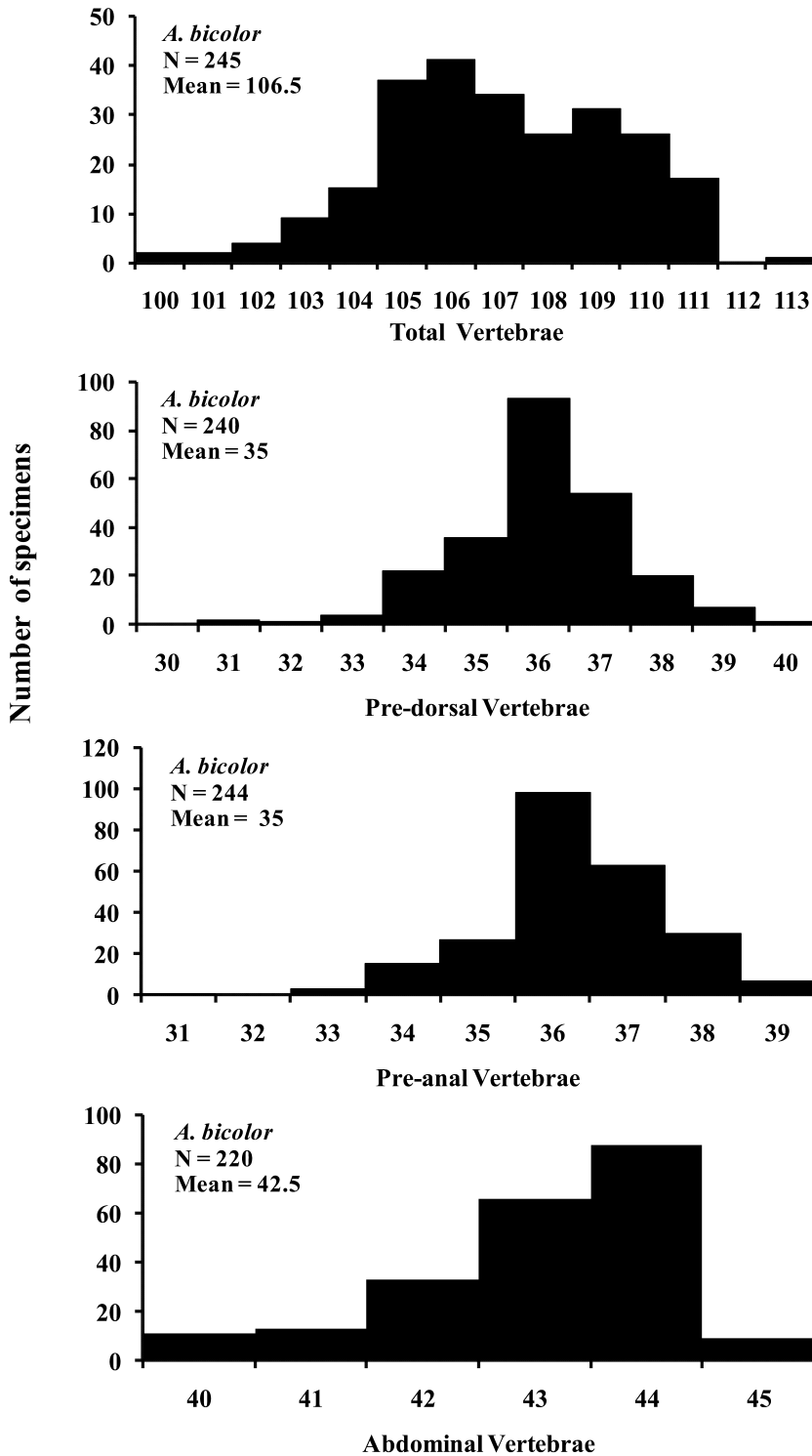


Fig. 3. Frequency distribution of total vertebrae, *pre-dorsal* vertebrae, *pre-anal* vertebrae, and *abdominal* vertebrae of the tropical short-finned eels, *Anguilla bicolor*, collected in the Indonesian Waters.

**Table 1.** Internal and external morphological characters of the two species and subspecies of tropical short-finned eels *A. bicolor bicolor* and *A. bicolor pacifica* collected in the Indonesian Waters

Species and subspecies	Sampling location (N)	Character	TL	PDL	PAL	ADL	TV	PDV	PAV	ADV
<i>A. bicolor bicolor</i>										
Krueng Aceh Estuary			Range	51.00	19.00	20.00	1.00			
(1)										
Air Kertaun Estuary			Range	49.50 ~ 54.00	18.00 ~ 20.50	18.00 ~ 21.00	-0.50 ~ 1.00			
(11)			Mean ± SD	52.14 ± 1.19	19.00 ± 0.75	20.00 ± 0.89	0.27 ± 0.52			
Cibaliung Estuary			Range	50.00 ~ 57.00	17.00 ~ 21.40	18.50 ~ 22.00	-0.50 ~ 2.00	105 ~ 113	33 ~ 39	33 ~ 39 -3 ~ 4
(100)			Mean ± SD	53.12 ± 1.55	19.18 ± 0.85	20.09 ± 0.72	0.91 ± 0.58	109.08 ± 1.50	36.00 ± 1.17	36.25 ± 1.32 1.10 ± 1.26
Citanduy Estuary			Range	48.00 ~ 50.00	18.00 ~ 20.00	18.00 ~ 21.50	0.00 ~ 1.50			
(3)			Mean ± SD	49.00 ± 1.00	19.33 ± 1.15	20.00 ± 1.80	0.67 ± 0.76			
Pacitan Estuary			Range	50.00 ~ 54.00	18.00 ~ 22.30	18.50 ~ 22.50	0.00 ~ 1.00			
(4)			Mean ± SD	51.75 ± 1.71	19.70 ± 1.93	20.08 ± 1.71	0.38 ± 0.49			
<i>A. bicolor pacifica</i>										
Palu Estuary			Range	45.00 ~ 52.00	17.00 ~ 20.00	17.00 ~ 21.00	-1.00 ~ 1.00	103 ~ 110	34 ~ 39	34 ~ 38 -3 ~ 2
(37)			Mean ± SD	49.19 ± 1.75	18.79 ± 0.98	19.03 ± 0.78	0.23 ± 0.71	106.18 ± 1.83	36.25 ± 1.43	36.32 ± 1.16 0.21 ± 1.32
Dumoga Estuary			Range	45.00 ~ 53.00	17.00 ~ 21.00	18.00 ~ 21.00	-1.00 ~ 1.00	103 ~ 109	34 ~ 39	33 ~ 39 -3 ~ 3
(57)			Mean ± SD	49.12 ± 1.85	18.85 ± 0.86	18.92 ± 0.78	0.07 ± 0.39	105.77 ± 1.31	36.46 ± 1.07	36.65 ± 0.97 0.04 ± 1.05
Poigar Estuary			Range	46.00 ~ 54.50	17.00 ~ 21.00	17.00 ~ 21.00	-1.00 ~ 1.00	95 ~ 108	21 ~ 39	27 ~ 39 -3 ~ 3
(46)			Mean ± SD	49.87 ± 1.76	18.84 ± 0.96	19.11 ± 0.95	0.27 ± 0.56	104.72 ± 2.54	35.29 ± 2.90	36.04 ± 1.76 0.35 ± 1.06
Bone Bolango Estuary			Range	48.00 ~ 52.50	18.00 ~ 20.00	19.00 ~ 20.50	-0.50 ~ 1.00	101 ~ 104	33 ~ 38	33 ~ 38 0 ~ 1
(4)			Mean ± SD	50.38 ± 1.89	19.13 ± 0.85	19.50 ± 0.71	0.38 ± 0.63	103.00 ± 1.41	34.25 ± 2.50	34.50 ± 2.38 0.75 ± 0.50
Akelamo Estuary			Range	46.00 ~ 50.00	13.00 ~ 19.00	14.00 ~ 20.00	-0.50 ~ 1.00	105 ~ 110	33 ~ 37	34 ~ 36 -3 ~ 1
(5)			Mean ± SD	48.08 ± 1.50	17.30 ± 2.44	18.00 ± 2.35	0.70 ± 0.65	107.83 ± 1.94	35.17 ± 1.47	35.00 ± 0.89 -0.17 ± 1.60
Panni Estuary			Range	46.00 ~ 55.00						
(4)			Mean ± SD	49.50 ± 4.36						

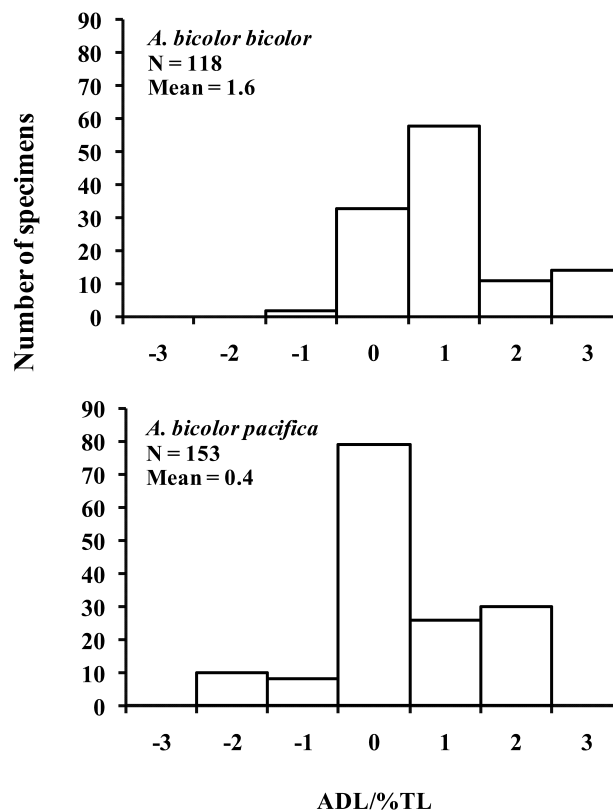


**Adjustment subspecies *Anguilla bicolor bicolor* and *Anguilla bicolor pacifica***

In the present study, subspecies discrimination of *A. bicolor* could not be done based on morphological analyses alone. The species had to show a wide range of ADL/%TL and ADV from -2 to 3 and -3 to 4, respectively. Therefore, sampling location is useful determining subspecies where their ranges do not overlap (Ege, 1939; Jespersen, 1942; Aoyama *et al.*, 2001, 2003, and 2007; Watanabe *et al.*, 2005a, b; Sugeha *et al.*, 2001a, b and 2008a, b, c). In the present study, all specimens of *A. bicolor* collected in the estuaries of Sumatera Island and Jawa Island were assigned to *A. bicolor bicolor* (N=119) while all specimens of collected in the estuaries of Sulawesi Island, Halmahera Island, and Papua Island were assigned to *A. bicolor pacifica* (N=153).

Total body length of *A. bicolor bicolor* glass eels ranged from 48 to 57mm ( $52.86 \pm 1.67$ ) while total body weight was ranged from 0.10 to 0.18gr ( $0.14 \pm 0.02$ ). Total body length of *A. bicolor pacifica* glass eels was 45 to 55mm ( $49.54 \pm 1.99$ ) while total body weight ranged from 0.6 to 0.19gr ( $0.11 \pm 0.02$ ). In general *A. bicolor bicolor* was bigger than *A. bicolor pacifica* in both total length and body weight. Complete morphological character of the body measurements and vertebrae counts of each subspecies in each sampling location was seen in Table 1.

*Ano-dorsal* length as a percentage of total length (ADL/%TL) overlapped between the two subspecies.

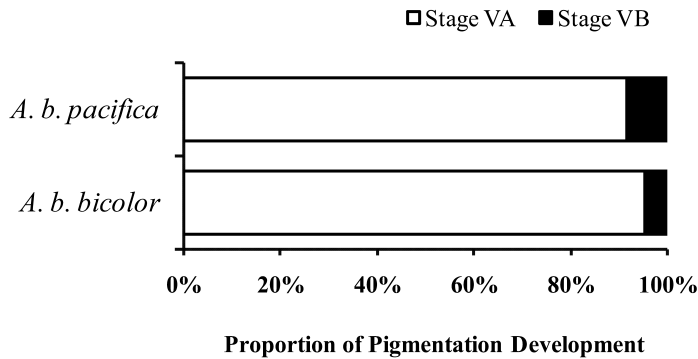


**Fig. 4.** Frequency distribution of *ano-dorsal* length as a percent of total length (ADL/%TL) of the two tropical short-finned eels species and subspecies (*A. bicolor bicolor* and *A. bicolor pacifica*) from Indonesian waters.

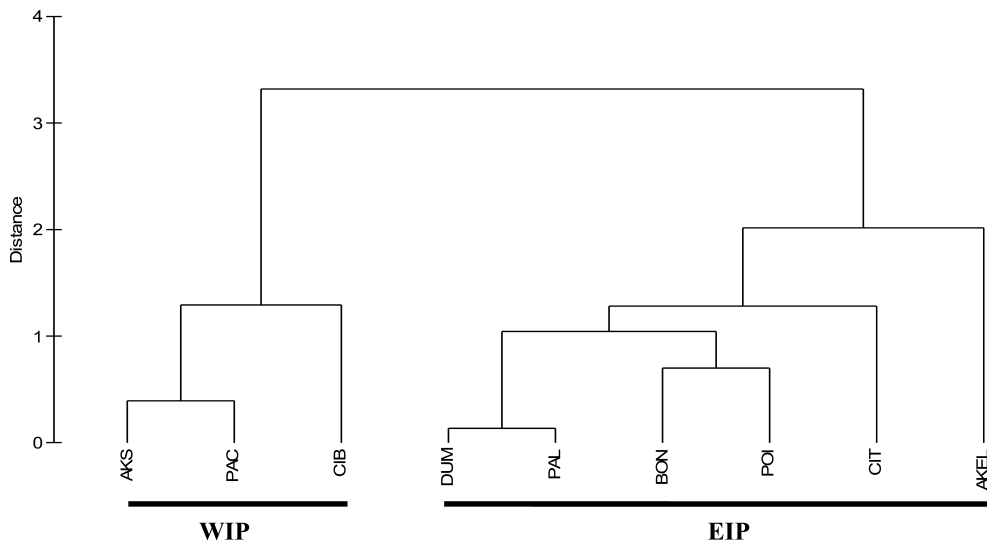
Remarks: Negative and positive numbers expressed the dorsal fin origin located behind and in front of the anal fin origin, respectively. Zero (0) number expressed the dorsal fin origin and the anal fin origin located symmetrically.

*Anguilla bicolor bicolor* was about -1 to 3 ( $1.6 \pm 1.15$ ) while *A. bicolor pacifica* was about -2 to 2 ( $0.4 \pm 1.13$ ) (Figure 4). An overlap occurred from -1 to 2 of ADL/%TL. Number of ano-dorsal vertebrae (ADV) also overlapped between the two subspecies. *Anguilla bicolor bicolor* was about -3 to 4 ( $1.10 \pm 1.26$ ) while *A. bicolor pacifica* was about -3 to 2 ( $1.10 \pm 1.26$ ). An overlap occurred from -3 to 2 of ADV (Table 1).

*Anguilla bicolor bicolor* and *A. bicolor pacifica* were dominated by pigmentation development stage VA (95.45% and 95%, respectively) (Figure 5). They were in the early stage of pigmentation



**Fig. 5.** Proportion of pigmentation development of the two subspecies of *A. bicolor* collected in the Indonesian Waters



**Fig. 6.** Cluster analyses of morphometric data of the tropical short-finned eels, *Anguilla bicolor*, separated the species in two clades. The first clade consists of the eels collected from Air Kertaun (AKS=2), Cibaliung (CIB=3) and Pacitan (PAC=5) estuaries while the second clade consists of the eels collected from Citanduy (CIT=4); Palu (PAL=6), Dumoga (DUM=7), Poigar (POI=8), Bone Bolango (BON=9) and Akelamo (AKEL=10) estuaries. The first clade reflected Western Indonesian Population (WIP) of *A. bicolor* with dominancy of *A. bicolor bicolor* and the second clade reflected Eastern Indonesian Population (EIP) of *A. bicolor* with dominancy of *A. bicolor pacifica*. Remarks: Data from Krueng Aceh (KRA=1) and Pami estuaries (PAM=11) were not included in the analyses since the number of specimen is only one. The number after abbreviation corresponds to the number of location in the Figure 1.

development and near the end of metamorphosis when collected. It is suggested that the two subspecies experience similar physiological conditions of pigmentation development during their coastal migration before reaching the estuaries of Indonesian continents.

### ***Population structure of *Anguilla bicolor****

Cluster analyses separated the tropical short-finned eels into two major clades (Figure 6). The first clade consists of *A. bicolor bicolor* from Cibaliung, Air Kertaun, and Pacitan River estuaries. The second clade consists of *A. bicolor pacifica* from Palu, Dumoga, Poigar, Bone Bolango, and Akelamo River estuaries. Interestingly, *A. bicolor bicolor* from Citanduy River estuary (Jawa Island) was nested into the second clade.

## **Discussion**

### ***Species dan Subspecies discrimination and distribution***

This study is the first to study the discrimination of the two tropical short-finned eels, *A. bicolor bicolor* and *A. bicolor pacifica*, based on both morphology and geography data analyses of the glass eels from the bio-geographically complex of Indonesian waters. *Anguilla bicolor bicolor* and *A. bicolor pacifica* overlap morphologically. However, they could be recognized statistically supporting their reported range of distribution in Indonesian waters. Species and subspecies discrimination in the eel genus *Anguilla* has become one of the most interesting topics for study on eel biology this decade. Aoyama *et al.* (1999) concluded that morphological study could not discriminate between *A. celebesensis* and *A. interioris* and suggested that mitochondrial DNA sequences are required to solve the problem. Dijkstra and Jellyman (1999) also found a problem validating the subspecies classification of *A. australis* (*A. australis australis* and *A. a. schmidtii*) in Australia and New Zealand and concluded that subspecies designation were justified. However, Watanabe *et al.* (2006) confirmed that considerable morphological differences exist between those two subspecies.

Those studies suggest that both morphological and genetic studies, as well as ecological study are required to identify *Anguilla* species and subspecies. The present study used both of morphological and ecological information to study the tropical short-finned eel, *A. bicolor*. However, in the future genetic study is required to validate our species and subspecies identifications. Previous study on the complete DNA mitochondrial genome of the species successfully discriminate the two subspecies, *A. bicolor bicolor* and *A. bicolor pacifica* (Minegishi *et al.*, 2001). However, those authors used specimens chiefly from outside the Indonesian waters, or from more subtropical area.

Following the study by Sugeha *et al.* (2008a b, c), this study is re-viewing geographic distribution of *A. bicolor* in the Indonesian waters since Ege (1939) and Jespersen (1942) for more than 50 years ago. In the present study it was found that *A. bicolor* were inhabit in the Sumatera Island (Krueng Aceh, Batang Antokan, and Air Kertaun Estuaries), Jawa Island (Cibaliung, Citanduy, Progo, and Pacitan Estuaries), Sulawesi Island (Palu, Poigar, Dumoga, and Bone Bolonago Estuaries), Halmahera Island (Akelamo Estuaries), and Papua Island (Pami Estuaries). *Anguilla bicolor pacifica* has been reported from Philippine waters (Jamandre *et al.*, 2007) and the western Pacific Ocean (Aoyama *et al.*, 1999). *Anguilla bicolor bicolor* was reported from South Africa and Myanmar (Watanabe *et al.*, 2005); Seychelles, Madras, and North Australian (Ege, 1939); Reunion (Ege, 1939; Watanabe *et al.*, 2005; Robinet *et al.*, 2002); Madagascar (Ege, 1939; Watanabe *et al.*, 2005), and Philippine Islands (Jamandre *et al.*, 2007). Those studies suggested that these two subspecies are mainly found in tropical waters. However, the present study strongly suggested that *A. bicolor* is mainly distributed in the tropical region of Indonesian waters. In detail, *A. bicolor bicolor* dominated in the western Indonesian waters while *A. bicolor pacifica* dominated in the center and eastern Indonesian waters. Moreover, it was proven that the Indonesian waters as the center of distribution and dispersal of many

anguillid eel species (Sugeha *et al.*, 2008a, b, c).

***Population study of A. bicolor and its implication to migratory history and spawning ecology of the species in the Indonesian Waters***

In the present study, the two subspecies of *A. bicolor* was separated into two clade, *i.e.* western Indonesian clade of *A. bicolor bicolor* and eastern Indonesian clade of *A. bicolor pacifica*. However *A. bicolor bicolor* from Citanduy River of western Indonesia nested in the second clade of eastern Indonesia. There are two possible explanations. First, miss identification such that specimens from Citanduy River actually were *A. bicolor pacifica*. If the specimens from Citanduy River were *A. bicolor pacifica*, then morphological data and range of geographic distribution cannot be used for subspecies identification of *A. bicolor* from Indonesian waters and that genetic identification should be applied. The occurrence of gene flow of the species and subspecies in the Indonesian waters might be an appropriate reason for answering the problem.

Second, if the identification was correct there may be multiple populations of *A. bicolor* in central Indonesian waters where the two subspecies overlap. Multiple populations of tropical anguillid eels have been reported in the tropical giant mottled eel *A. marmorata* (Minegishi *et al.*, 2008). The authors reported the occurrence of *A. marmorata* populations from the North and South Pacific in Ambon Island (Indonesia) based on genetic population study. In a recent study (Watanabe *et al.*, 2005) on the population structure of *A. bicolor bicolor* based on morphology and genetic analyses of specimens from Indian Ocean, including the western Indonesian region, it was suggested that there is no population of *A. bicolor bicolor* in the eastern and western side Indian Ocean. However, a more recent study reported the occurrence of *A. bicolor bicolor* in Philippine waters that life sympatric with *A. bicolor pacifica* and *A. marmorata* (Jamandre *et al.*, 2007). The phenomenon may be possible to occur in the Indonesian region since Philippine waters directly connected with the Sulawesi and Maluku Seas of Indonesian in conjunction with the Indonesian Trough flow. If this idea is correct then it might answer why *A. bicolor bicolor* from Citanduy estuary nested with eastern Indonesian clade of *A. bicolor pacifica*. Glass eels might be transported from western to eastern region of Indonesian waters via Indian Ocean tidal propagation (Tomascik *et al.*, 1997) that is regulated by tropical monsoons in the Indonesian region. The Indian Ocean tidal propagation influences tidal condition in the western Indonesian region and might be affecting transport and mixing processes in the Inter-Tropical Convergence Zone (ITCZ) of the Indonesian region (Hatayama *et al.*, 1996; Tomascki *et al.*, 1997). If true, then *A. bicolor pacifica* may appear in the first clade of western Indonesian waters after transported by Indonesian Trough flow.

Interestingly, Sugeha *et al.* (2008c) reported dominance of *A. bicolor pacifica* glass eels among *Anguilla* species during inshore migration in the estuary of Palu River (western Sulawesi Island) located in front of Makassar Strait. This dominance is unusual because previous reports suggested that the species always was the least abundant of tropical eel species in north Sulawesi Island (Arai *et al.*, 1999; Sugeha *et al.*, 2001a and 2008b), in central Sulawesi Island (Sugeha *et al.*, 2001b) and more eastern regions of Indonesian waters (Sugeha *et al.*, 2008a). A greater catch of *A. bicolor pacifica* in western Sulawesi Island of central Indonesian waters might be evidence of isolated population there that is ecologically separated from eastern Indonesian population. Alternatively, central Indonesian *A. bicolor pacifica* may come from eastern Indonesian population during larvae migration through Indonesian Trough flow of the Makassar Strait. The migration of *A. bicolor pacifica* larvae from eastern to western Indonesian waters is worth considering since *A. interioris* that usually occur in eastern Papua New Guinea have been found in waters around North Sulawesi Island (Sugeha *et al.*, 2008b) and west Sumatera Island (Aoyama *et al.*, 2007; Sugeha *et al.*, 2008a). Also *A. obscura* and *A. megastoma* that usually are found in western Papua Island but has been found in the waters around Halmahera and Sulawesi Islands (Sugeha *et al.*, 2008a). Genetic study of population structure for the

tropical short-finned eels in the Indonesian waters is required to clarify the phenomenon.

Our results support previous studies that two *A. bicolor* subspecies were separated geographically and biologically, *i.e.* western Indonesian clade of *A. bicolor bicolor* which may be derived from the Indian Ocean and eastern Indonesian clade of *A. bicolor pacifica* which may be derived from the Pacific Ocean. If this idea is correct, then this study supports the hypothesis that *A. bicolor bicolor* may spawn in the Indian Ocean (Jespersen, 1942; Kuroki *et al.*, 2006; Aoyama *et al.*, 2007) and *A. bicolor pacifica* may spawn in the Pacific Ocean (Arai *et al.*, 1999d; Aoyama *et al.*, 1999). However, the anomaly of *A. bicolor bicolor* from Citanduy estuary and the dominance of *A. bicolor pacifica* in Palu estuary might indicate relatively close spawning ground of the two subspecies from their recruitment area in the central Indonesian waters.

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